



Technical Bulletin

PowerPlex® 5-Dye Matrix Standards, 3100/3130

INSTRUCTIONS FOR USE OF PRODUCTS DG4700.

Note: These matrix fragments are compatible with the ABI PRISM® 3100 and 3100-*Avant* and Applied Biosystems 3130, 3130xl, 3500 and 3500xL Genetic Analyzer.

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PowerPlex® 5-Dye Matrix Standards, 3100/3130

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1. Description

Proper generation of a spectral calibration file is critical to evaluate multicolor systems with the ABI PRISM® 3100 and 3100-Avant and Applied Biosystems 3130, 3130xL, 3500 and 3500xL Genetic Analyzers. The PowerPlex® 5-Dye Matrix Standards, 3100/3130^(a-c), consists of DNA fragments labeled with five different fluorescent dyes: one tube contains a DNA fragment labeled with fluorescein, one tube contains a DNA fragment labeled with JOE, one tube contains a DNA fragment labeled with TMR-ET, one tube contains a DNA fragment labeled with CXR-ET, and one tube contains a DNA fragment labeled with CC5.

1. Description (continued)

These matrix fragments are mixed and used on the ABI PRISM® 3100 or 3100-*Avant* or Applied Biosystems 3130, 3130*xl*, 3500 or 3500*xL* Genetic Analyzer to perform a spectral calibration on a specified dye set. The spectral calibration should be performed on dye set G5. Once generated, this file is applied during sample detection to calculate the spectral overlap between the five different dyes and separate the raw fluorescent signals into individual dye signals. The PowerPlex® 5-Dye Matrix Standards, 3100/3130, was developed for use with the 5-dye PowerPlex® Systems. A matrix should be generated for each individual instrument. Protocols for operation of the ABI PRISM® 3100 or 3100-*Avant* or Applied Biosystems 3130, 3130*xl*, 3500 or 3500*xL* Genetic Analyzer should be obtained from the manufacturer.

2. Product Components and Storage Conditions

Product	Size	Cat.#
PowerPlex® 5-Dye Matrix Standards, 3100/3130	25µl (each dye)	DG4700

Not for Medical Diagnostic Use. Includes:

- 25µl Fluorescein Matrix, 5-Dye (3100/3130)
- 25µl JOE Matrix, 5-Dye (3100/3130)
- 25µl TMR-ET Matrix, 5-Dye (3100/3130)
- 25µl CXR-ET Matrix, 5-Dye (3100/3130)
- 25µl CC5 Matrix, 5-Dye (3100/3130)
- 1.25ml Nuclease-Free Water


Storage Conditions: Store all components at -20°C in a nonfrost-free freezer. The matrix standards fragments are light-sensitive and must be stored in the dark. We strongly recommend that the matrix standards be stored with post-amplification reagents and used separately with different pipettes, tube racks, etc.


Do not store the diluted matrix standard fragments.

3. Instrument Preparation and Spectral Calibration Using the Applied Biosystems 3500 and 3500xL Genetic Analyzers

Materials to Be Supplied by the User


- 95°C dry heating block, water bath or thermal cycler
- crushed ice or ice-water bath
- centrifuge compatible with 96-well plates
- aerosol-resistant pipette tips
- 3500/3500xL capillary array, 36cm
- performance optimized polymer 4 (POP-4™) in a pouch for the 3500 or 3500xL
- anode buffer container with 1X buffer
- cathode buffer container with 1X buffer
- conditioning reagent pouch for the 3500 or 3500xL
- MicroAmp® optical 96-well plate and septa
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

 The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at -20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

 Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take the necessary precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

For additional information on performing spectral calibration, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*.

3.A. Matrix Sample Preparation

 There may be instrument-to-instrument variation in the sensitivity of detection. The dilutions described here may need to be optimized in individual laboratories, depending on the sensitivity of each Applied Biosystems 3500 or 3500xL Genetic Analyzer.

1. Thaw the matrix standards on ice. Mix each matrix standard by vortexing each tube for 5–10 seconds prior to use. Do not centrifuge the matrix standards as this may cause the DNA to be concentrated at the bottom of the tube. Place on ice.
2. **Initial dilution of concentrated fragments:** Before combining the matrix standards, dilute the individual matrix standards 1:10 in Nuclease-Free Water, as described below. Vortex for 5–10 seconds to mix. Place on ice.

Component	Fluorescein	JOE	TMR-ET	CXR-ET	CC5
Concentrated Dye	5µl	5µl	5µl	5µl	5µl
Nuclease-Free Water	45µl	45µl	45µl	45µl	45µl

3.A. Matrix Sample Preparation (continued)

3. **Fragment mix (using 1:10 dilutions of matrix standards):** After the initial dilution in Step 2, combine the 1:10 dilution of each matrix standard as directed below. Vortex for 5–10 seconds to mix.

Component	Volume
Hi-Di™ formamide	665µl
Fluorescein from initial dilution	7.0µl
JOE from initial dilution	7.0µl
TMR-ET from initial dilution	7.0µl
CXR-ET from initial dilution	7.0µl
CC5 from initial dilution	7.0µl

4. On the Applied Biosystems 3500xL Genetic Analyzer, only wells A1 to H3 of the 96-well plate are used for spectral calibration. Load 25µl of the fragment mix prepared in Step 3 into each of the 24 wells. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles.

On the Applied Biosystems 3500 Genetic Analyzer, only wells A1 to H1 of the 96-well plate are used for spectral calibration. Load 25µl of the fragment mix prepared in Step 3 into each of the 8 wells. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles.

5. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.
6. Place the plate in the 3500 series 96-well standard plate base, and cover with the plate retainer. Place the plate assembly in Position A on the autosampler with the labels facing you.

3.B. Instrument Preparation

1. Set the oven temperature to 60°C, then select the Start Pre-Heat icon at least 30 minutes prior to the first injection to preheat the oven (Figure 1).



Figure1. The Dashboard. The arrow highlights the pre-heat options.

3.B. Instrument Preparation (continued)

2. To perform a spectral calibration with the Promega 5-dye chemistry, a new dye set should be created. If a new dye set was created previously, proceed to Section 3.B, Step2.c.
 - a. To create this new dye set, navigate to the Library, highlight “Dye Sets” and select “Create”.
 - b. The Create a New Dye Set tab will appear (Figure 2). Name the Dye Set, select “Matrix Standard” for the Chemistry and select “G5 Template” for the Dye Set Template. Select “Save”.

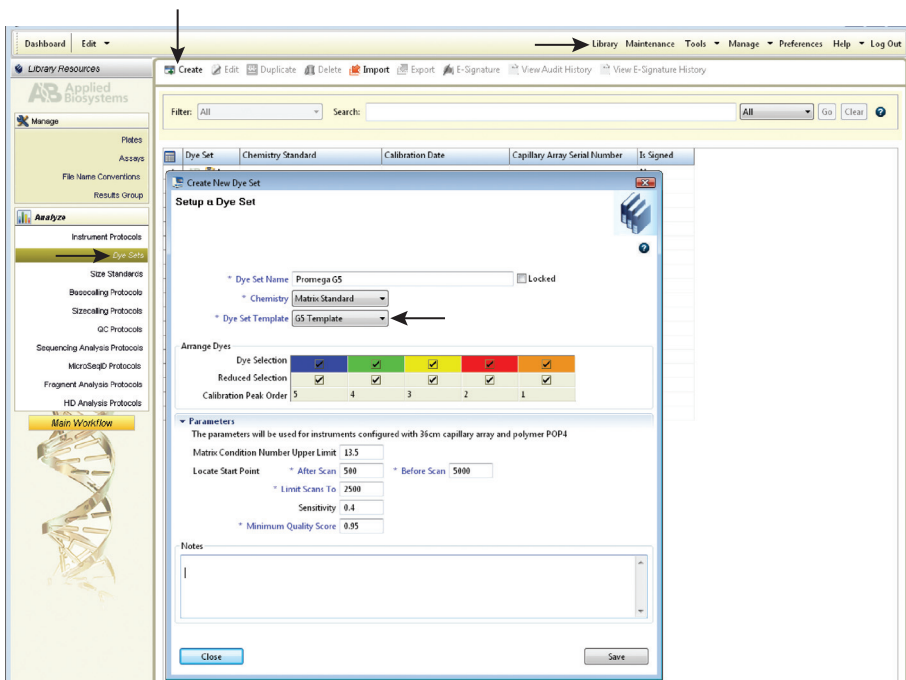


Figure 2. Create New Dye Set.

- c. To perform the spectral calibration with the Promega 5-dye chemistry, go to the Maintenance tab, select "Spectral", and under the Calibration Run tab, choose the appropriate fields: Choose "Matrix Standard" from the Chemistry Standard pull-down menu and the new Promega 5-dye set created in Step 2b (i.e., Promega G5) from the Dye Set pull-down menu (Figure 3).
- d. Select "Start Run".

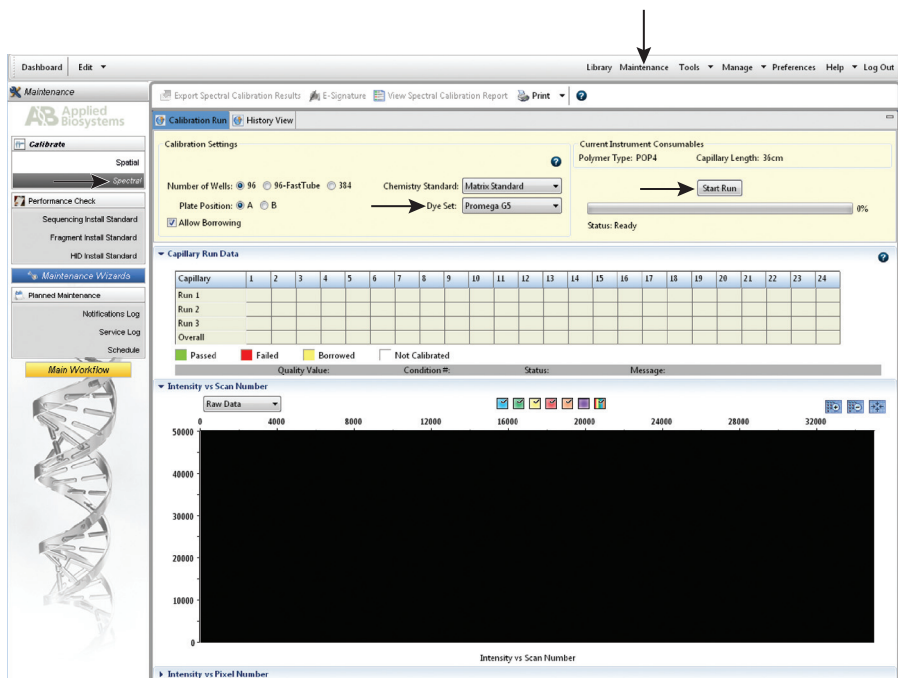


Figure 3. Calibration Run.

3.B. Instrument Preparation (continued)

3. If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display (Figure 4), and choose either “Accept” or “Reject”.

Note: Refer to the *3500 Series Data Collection Software Version 1.0 HID User Manual* for the criteria recommended by Applied Biosystems when accepting or rejecting a spectral calibration.

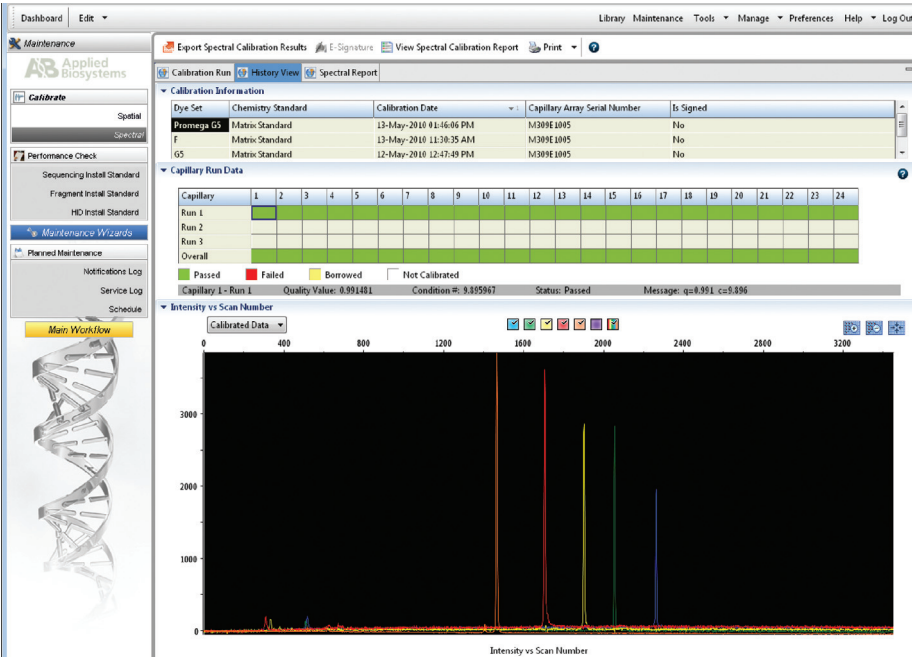




Figure 4. The Capillary Run Data display.

4. Instrument Preparation and Spectral Calibration Using Data Collection Software, Version 2.0 or Version 3.0 (ABI PRISM® 3100 and 3100-*Avant* and Applied Biosystems 3130 and 3130xl Genetic Analyzers)

Materials to Be Supplied by the User

- 95°C dry heating block, water bath or thermal cycler
- crushed ice or an ice-water bath
- centrifuge compatible with 96-well plates
- 3100 or 3130 capillary array, 36cm
- performance optimized polymer 4 (POP-4™) for the 3100 or 3130
- 10X genetic analyzer buffer with EDTA
- MicroAmp® optical 96-well plate
- aerosol-resistant pipette tips
- Hi-Di™ formamide (Applied Biosystems Cat.# 4311320)

 The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at -20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

 Formamide is an irritant and a teratogen; avoid inhalation and contact with skin. Read the warning label, and take appropriate precautions when handling this substance. Always wear gloves and safety glasses when working with formamide.

4.A. Matrix Sample Preparation

There may be instrument-to-instrument variation in the sensitivity of detection. The dilutions described here may need to be optimized in individual laboratories, depending on the sensitivity of each ABI PRISM® 3100 or 3100-*Avant* or Applied Biosystems 3130 or 3130xl Genetic Analyzer. The optimal dilution may differ for each dye fragment. You also may need to adjust injection time or voltage in Section 4.B to achieve a passing spectral calibration. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU are required.

The same plate of matrix standards can be re-injected up to four times. To re-inject the same matrix standards plate, add an injection by selecting “Plate Manager”, then “Edit”. Select “Edit” again in the top left corner of the window, then select “Add sample run”. Injection time and voltage may require optimization to obtain peak heights above 750RFU and below the saturation point.

1. Thaw the matrix standards on ice. Mix each matrix standard by vortexing for 5–10 seconds prior to use. Do not centrifuge the matrix standards as this may cause the DNA to be concentrated at the bottom of the tube. Place on ice.

4.A. Matrix Sample Preparation (continued)

2. **Initial dilution of concentrated fragments:** Before combining the matrix standards, dilute the individual matrix standards 1:10 in Nuclease-Free Water, as described below. Vortex for 5–10 seconds to mix. Place on ice.

Component	Fluorescein	JOE	TMR-ET	CXR-ET	CC5
Concentrated Dye	5µl	5µl	5µl	5µl	5µl
Nuclease-Free Water	45µl	45µl	45µl	45µl	45µl

3. **Fragment mix (using 1:10 dilutions of matrix standards):** After the initial dilution in Step 2, combine the 1:10 dilutions as directed below. Vortex for 5–10 seconds to mix.

Component	Volume
Hi-Di™ formamide	475µl
Fluorescein from initial dilution	5.0µl
JOE from initial dilution	5.0µl
TMR-ET from initial dilution	5.0µl
CXR-ET from initial dilution	5.0µl
CC5 from initial dilution	5.0µl

4. On the ABI PRISM® 3100 and Applied Biosystems 3130xl Genetic Analyzers, 16 wells are used for spectral calibration on 16 capillaries (wells A1 through H2 of a 96-well plate). Load 25µl of fragment mix prepared in Step 3 into each of the 16 wells. Briefly centrifuge the plate to remove bubbles.

On the ABI PRISM® 3100-*Avant* and Applied Biosystems 3130 Genetic Analyzers, four wells are used for spectral calibration on four capillaries (wells A1 through D1 in a 96-well plate). Load 25µl of fragment mix into each of the four wells. Briefly centrifuge the plate to remove any bubbles.

5. Denature samples at 95°C for 3 minutes, then immediately chill on crushed ice or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

4.B. Instrument Preparation

We have found that the use of fresh polymer and new capillary array results in an optimal spectral.

Representative raw data are shown in Figure 5. Another example of a passing spectral calibration in the Spectral Viewer is shown in Figure 6.

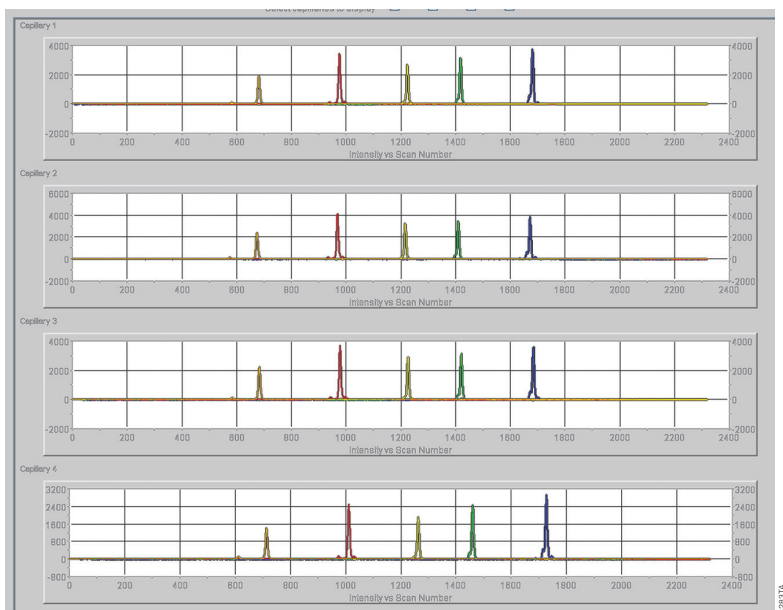


Figure 5. Representative data for the PowerPlex® 5-Dye Matrix Standards, 3100/3130, on the Applied Biosystems 3130 Genetic Analyzer using Foundation Data Collection Version 3.0. Figure shows the CC5, CXR-ET, TMR-ET, JOE and fluorescein peaks in the raw data profile from each of the four capillaries in the Capillaries Viewer.

4.B. Instrument Preparation (continued)

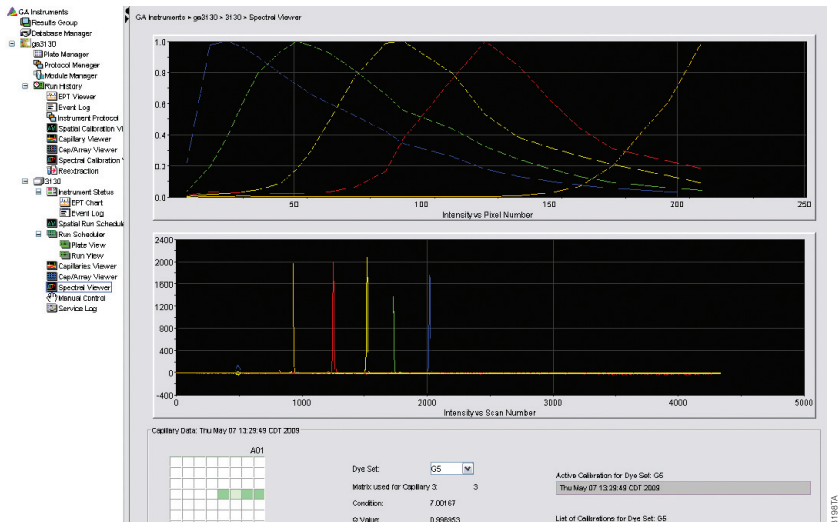


Figure 6. Representative data for the PowerPlex® 5-Dye Matrix Standards, 3100/3130, on the Applied Biosystems 3130 Genetic Analyzer using data collection software, version 3.0. Figure shows the CC5 (orange), CXR-ET (red), TMR-ET (yellow), JOE (green) and fluorescein (blue) peaks in the Spectral Viewer from one of the four capillaries.

1. Prepare matrix samples as previously described in Section 4.A.

Note: Differences in instrument sensitivity may result in peak imbalance or reduced peak height of the matrix standards. You may need to adjust injection time or voltage to achieve a passing spectral calibration. You also may need to prepare a new plate and adjust the dilution of individual matrix standards. Peak heights in the range of 1,000–4,000RFU are ideal. Peak heights above 750RFU and below the saturation point of the instrument are required.

2. Perform the spectral calibration as described in the ABI PRISM® 3100 or 3100-Avant or Applied Biosystems 3130 or 3130xl Genetic Analyzer User's Manual with the following modifications:
 - a. In the Module Manager, select "New." Select "Spectral" in the Type drop-down list, and select "Spect36_POP4" in the Template drop-down list. Confirm or change the following settings:

Inj. kV:	1.2
Inj. Secs:	12
Data Delay Time:	400
Run time:	700 seconds

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- b. Create a new name for the run module, then select "OK".
 - c. In the Protocol Manager, under Instrument Protocols select "New". Type a name for your protocol.

Make the following selections in the Protocol Editor:

- "Spectral" in the Type drop-down list
- "G5" in the DyeSet drop-down list
- "POP4" for the polymer
- "36" in the Array Length drop-down list
- "Matrix Standard" for the chemistry
- Select the spectral module you created in the previous step in the Run Module drop-down list.

Finally, select "Edit Parameters", and make the following modifications:

- Change the lower condition bound to 4.0, and change the upper condition bound to 12.0.
- Confirm that the Minimum Quality Score is 0.95

Select "OK" in the Edit Parameters window, and select "OK" in the Protocol Editor.

Note: The condition number ("C value") obtained when generating a spectral calibration will vary with the instrument. After obtaining a spectral calibration that performs acceptably, the condition bounds range in the previous step may be narrowed to more critically evaluate C values for subsequent spectral calibrations.

- d. In the Plate Manager, create a new plate record as described in the instrument user's manual. In the dialog box that appears, select "Spectral Calibration" in the Application drop-down list, and select "96-well" as the plate type. Add entries in the owner and operator windows, name the plate and select "OK."
- e. In the spectral calibration plate editor dialog box, place sample names in the appropriate cells. In the Instrument Protocol column, select the protocol you created in Step 2.c. Ensure that this information is present for each row that contains a sample name. Select "OK."
- f. Run your plate as described in the instrument user's manual.
- g. Upon completion of the run, check the status of the spectral calibration in the Event Log window. For the ABI PRISM® 3100 and Applied Biosystems 3130xl Genetic Analyzers, we recommend that a minimum of 12 of the 16 capillaries pass calibration. For the ABI PRISM® 3100-Avant and Applied Biosystems 3130 Genetic Analyzers, we recommend that a minimum of three of four capillaries pass calibration. If fewer than the recommended numbers of capillaries pass, repeat the spectral calibration.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

5.A. Applied Biosystems 3500 and 3500xL Genetic Analyzers

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	Matrix standards were too dilute. Matrix samples that are too dilute will result in low peak heights, which may result in spectral calibration failure or bleedthrough or oversubtraction in other dye colors. Decrease the dilution of each fragment in Step 2 of Section 3.A.
	Matrix standards were too concentrated. Matrix samples that are too concentrated may result in spectral calibration failure or bleedthrough or oversubtraction in other dye colors. Increase the dilution of each fragment in Step 2 of Section 3.A.
	Samples were not denatured completely. Incomplete denaturation can cause extra peaks. Heat-denature samples for the recommended time, and cool on crushed ice or in an ice-water bath immediately prior to loading the capillary. Do not cool samples in a thermal cycler or -20°C freezer.

5.B. ABI PRISM® 3100 and 3100-Avant and Applied Biosystems 3130 and 3130xL Genetic Analyzers

Symptoms	Causes and Comments
Fewer than the recommended number of capillaries passed the spectral calibration	Peak heights for the matrix standards were too low. Increase the injection voltage or time. If the matrix peak heights are below 1,000RFU, decrease the dilution of each fragment in Step 2 of Section 4.A, and repeat the spectral calibration.
	Peak heights for the matrix standards were too high. Decrease the injection voltage or time. Matrix sample peak heights that are too high may result in spectral calibration failure. If matrix sample peak heights are too high (>5,000RFU), increase the dilution of each fragment in Step 2 of Section 4.A, and repeat the spectral calibration.
	For best spectral calibration results, use a fresh bottle of polymer, fresh buffer and water, and a capillary array with fewer than 100 injections.

Symptoms	Causes and Comments
All capillaries failed spectral calibration	<p>Monitoring fragment migration in the Capillaries Viewer during the spectral calibration run can provide information that will be useful for troubleshooting purposes. Re-inject the spectral calibration plate, and monitor the Capillaries Viewer during the run. Note any unusual peak formations, extremely high or low peak heights. Based on information obtained while watching the Capillary Viewer, it may be necessary to adjust the run conditions.</p> <p>Samples were degraded due to improper storage. Store matrix standards at -20°C and protected from light. Do not store in the freezer door, and do not store in a frost-free freezer.</p>

6. Related Products

Product	Size	Cat.#
PowerPlex® 18D System	200 reactions	DC1802
	800 reactions	DC1808
PowerPlex® ESX 16 System	100 reactions	DC6711
	400 reactions	DC6710
PowerPlex® ESX 17 System	100 reactions	DC6721
	400 reactions	DC6720
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® ESI 17 System	100 reactions	DC6781
	400 reactions	DC6780

Not for Medical Diagnostic Use.

Accessory Components

Product	Size	Cat.#
Nuclease-Free Water*	50ml	P1193
Water, Amplification Grade*	6,250µl (5 × 1,250µl)	DW0991
PowerPlex® 5-Dye Matrix Standards, 310**	50µl (each dye)	DG4600

*For Laboratory Use.

**Not for Medical Diagnostic Use.

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^(c)TMR-ET, CXR-ET and CC5 dyes are proprietary.

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